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STATE-OF-THE-ART REVIEW

Human transfer RNA modopathies: diseases caused by aberrations in transfer RNA modifications

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Keywords
RNA; RNA modification; translation; tRNA; tRNA modopathy

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The conventional symbols of modified nucleosides can be found at RNA Modification Database (https://mods.rna.albany.edu).

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tRNA molecules are post-transcriptionally modified by tRNA modification enzymes. Although composed of different chemistries, more than 40 types of human tRNA modifications play pivotal roles in protein synthesis by regulating tRNA structure and stability as well as decoding genetic information on mRNA. Many tRNA modifications are conserved among all three kingdoms of life, and aberrations in various human tRNA modification enzymes cause life-threatening diseases. Here, we describe the class of diseases and disorders caused by aberrations in tRNA modifications as 'tRNA modopathies'. Aberrations in over 50 tRNA modification enzymes are associated with tRNA modopathies, which most frequently manifest as dysfunctions of the brain and/or kidney, mitochondrial diseases, and cancer. However, the molecular mechanisms that link aberrant tRNA modifications to human diseases are largely unknown. In this review, we provide a comprehensive compilation of human tRNA modification functions, tRNA modification enzyme genes, and tRNA modopathies, and we summarize the elucidated pathogenic mechanisms underlying several tRNA modopathies. We will also discuss important questions that need to be addressed in order to understand the molecular pathogenesis of tRNA modopathies.

Introduction and definition of 'tRNA modopathy'

The precise and efficient translation of genetic information into proteins is essential for life. tRNA molecules function as adaptor molecules that translate transcribed genetic information in the form of mRNA into 20 amino acids that form proteins [1,2]. Protein synthesis occurs in the cytoplasm using hundreds of human cytoplasmic tRNA species, which are transcribed from more than 400 tRNA genes encoded in the nuclear chromosomes [3]. Protein synthesis also takes place within mitochondria, where 13 oxidative phosphorylation (OXPHOS) complex proteins are translated using 22 tRNAs transcribed from mitochondrial DNA [4].

tRNA molecules are composed of a highly conserved cloverleaf secondary structure, which consists of an acceptor stem, dihydrouridine (D) loop, D arm, anticodon loop, anticodon arm, variable loop, TΨC (T)

Abbreviations
ADAT1, tRNA-specific adenosine deaminase 1; CDK5RAP1, CDK5 regulatory subunit-associated protein 1; CDKAL1, CDK5 regulatory subunit-associated protein 1-like 1; CTU1, cytoplasmic tRNA 2-thiolation protein 1; DUS2L, dihydrouridine synthase 2-like; eIF2α, eukaryotic initiation factor 2 subunit alpha; ELP1, elongator complex protein 1; FTSJ1, ftsJ homolog 1; GTPBP3, GTP-binding protein 3; HIF1α, hypoxia-inducible factor 1-alpha; METTL1, methyltransferase-like 1; MTO1, mitochondrial translation optimization protein 1 homolog; NAD, nicotinamide adenine dinucleotide; NSUN2, NOP1/NOP2/Sun domain family member 2; PUS1, pseudouridine synthase 1; QTRT1, queuine tRNA-ribosyltransferase 1; SNP, single nucleotide polymorphism; THG1L, tRNA-histidine guanylyltransferase 1-like; TRIT1, tRNA isopentenytransferase 1; TRMT1, tRNA methyltransferase 1; WDR4, WD repeat-containing protein 4. The conventional symbols of modified nucleosides can be found at RNA Modification Database (https://mods.rna.albany.edu).

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loop, and T arm (Fig. 1A,B). tRNA molecules form an L-shaped tertiary structure via multiple hydrogen bonds between loops and helices. In addition to the characteristic L-shape, another structural feature of tRNAs is their chemically modified nucleosides (Fig. 1C). tRNA modifications are post-transcriptionally added to tRNA by specific modifying enzymes. By counting one chemical structure as one modification (e.g., pseudouridines at various positions incorporated by different enzymes are counted as one modification), we counted 43 types of known stable tRNA modifications that exist in humans. These modifications are incorporated by at least 73 human enzymes and partner proteins (including confirmed proteins and widely accepted candidates). Due to their importance in protein synthesis, the dysfunction and aberrant expression of more than 50 tRNA modification enzymes are known to be associated with human diseases. The diseases caused by aberrations in RNA modification were collectively named 'RNA modopathies' by the Tsutomu Suzuki group and our group [5]. Although several RNA modopathies occur due to aberrations in mRNA or rRNA modifications, in this review, we will focus on 'tRNA modopathies', which are the diseases and disorders caused by aberrations in tRNA modifications.

In this review, we provide a comprehensive compilation of human tRNA modification functions, tRNA modification enzymes, and tRNA modopathies and discuss the important questions that need to be addressed to elucidate the pathogenic molecular mechanisms underlying tRNA modopathies. To understand how tRNA modopathies are caused, it is essential to understand the chemical properties and molecular functions of tRNA modifications. The most important functions of tRNA modifications are tRNA stability regulation and codon recognition. Therefore, we will start by considering these two functions. In this review, we introduce insights that were derived mostly from the study of mammalian cells and animals. Many tRNA modifications, however, are conserved or sometimes functionally converged across the three domains of life. Please refer to other excellent reviews for general information on the codon table [6], anticodon modifications [7], modification-mediated tRNA stabilization [8], methylation [9], pseudouridine [10], and modification pathways [11].

Function of tRNA modifications: regulation of the physical and biochemical stability of tRNA

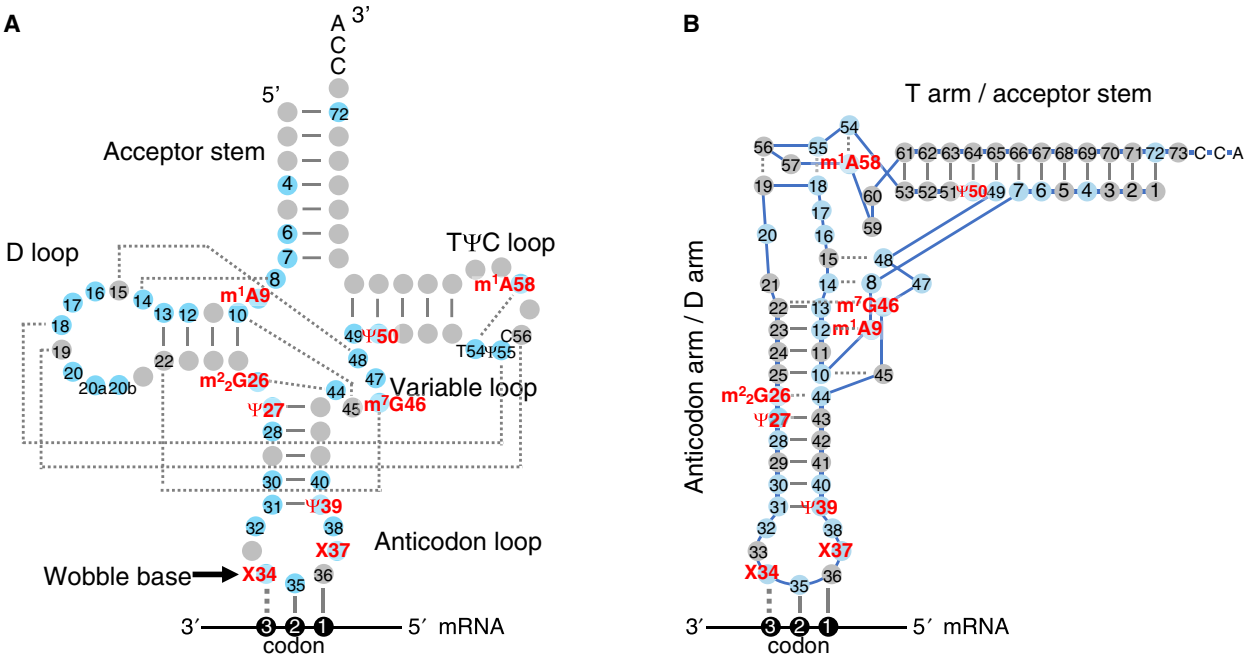
tRNA modifications regulate the stability of the tRNA structure in three ways: (a) stabilization of overall tRNA structure, (b) regulation of tRNA local structure, and (c) inhibition of RNase-mediated tRNA degradation.

Stabilization of overall tRNA structure

The overall tRNA structure can be compromised upon a loss of human tRNA modification, such as 1-methyladenosine (m^1A) at position 9 (m^1A9) and *N2,N2*-dimethylguanosine ($m^2{}_2G$) at position 26 ($m^2{}_2G26$). m^1A and $m^2{}_2G$ possess methyl groups in their Watson-Crick faces (Fig. 1C). Thus, m^1A9 and $m^2{}_2G26$ prevent the Watson-Crick base pairing of A–U and G–C, respectively. A classic example is human mitochondrial (mt) $tRNA^{Lys}$. An unmodified *in vitro* transcript of $tRNA^{Lys}$ forms a rod-like structure by making an aberrant A9-U64 base pair. The introduction of m^1A9, a single methyl group, leads to the disruption of the A9-U64 pair and enables the formation of a functional tRNA structure [12]. The correct tRNA structure is further stabilized by m^2G10, although m^2G10 alone cannot correct the tRNA structure [13]. The TRMT10C and HSD17B10 proteins cooperatively incorporate mt tRNA m^1A9 [14], and a mutation in either protein can result in mitochondrial dysfunction-associated disease that sometimes results in infantile death [15,16]. Many cytoplasmic tRNAs have m^1G and not m^1A at position 9. As the methyl group of m^1G also disrupts the G-C base pair, m^1G9 may also be involved in maintaining the tRNA structure, and experimental studies of this possibility are required.

Another example of a tRNA modification that stabilizes the overall tRNA structure is $m^2{}_2G26$ of the human cytoplasmic $tRNA^{Asn}$. Without $m^2{}_2G26$, an aberrant G26-C11 base pair forms in $tRNA^{Asn}$ and disrupts the tRNA structure. $m^2{}_2G26$ prevents the formation of that abnormal base pair and instead forms a hydrogen bond with A44 to stabilize the tRNA structure (Fig. 1A,B) [17]. $m^2{}_2G26$ is incorporated by tRNA methyltransferase 1 (TRMT1) [18,19], and mutations in TRMT1 result in microcephaly and intellectual disability [20].

A network of hydrogen bonds collectively contributes to maintaining the L-shaped tRNA tertiary structure (dotted lines in Fig. 1A,B). In the model case of yeast $tRNA^{Phe}$, such tertiary interactions include G18-Ψ55, G19-C56, and T(m^{5U})54-m^1A58 interactions. In addition, the tRNA structure is stabilized by base triplets, in which a canonical Watson-Crick base pair further interacts with a third base using the space in the major groove of the helix. Base triplets form between bases 25–10–45, 9–12–23, and 13–22–46 (Fig. 1B) [21]. Among these interactions, tRNA modifications of m^1A58 and m^7G46



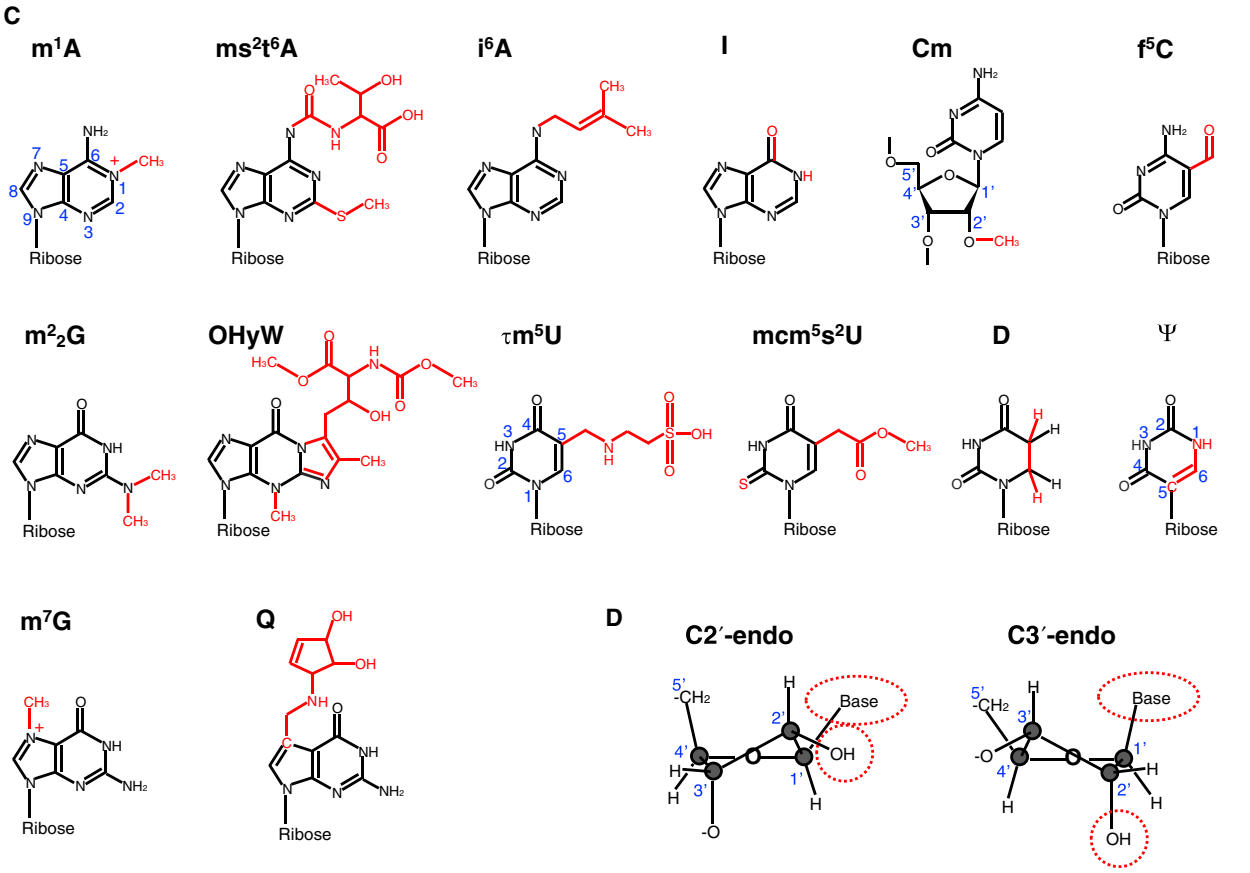


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Fig. 1. tRNA structure and tRNA modifications. (A) tRNA secondary structure depicted in a cloverleaf form. Nucleoside positions are numbered following conventional guidelines [223]. Red-lettered tRNA modifications affect tRNA structure in at least some tRNA species. Gray circle, unmodified nucleoside; blue circle, nucleoside known to be modified in at least one tRNA species; straight line between bases, Watson-Crick base pairs; dotted line between bases, hydrogen bond observed in yeast $tRNA^{Phe}$ tertiary structure [21]. (B) tRNA secondary structure depicted in the L-shape, based on the yeast $tRNA^{Phe}$ crystal structure [21,22]. Note that in the actual tertiary structure, a base-paired stem forms a helix. (C) Chemical structures of various tRNA modifications. (D) Ribose ring C2′-endo conformation and C3′-endo conformation. Note that in the C2′-endo form, the base and the 2′hydroxyl group are in close proximity, and Nm or xm^5s^2U modifications induce steric repulsion between the base and 2′hydroxyl group to favor the C3′-endo form. 1-methyladenosine (m^1A), ms^2t^6A (2-methylthio-*N6*-threonyl carbamoyladenosine), i^6A (*N6*-isopentenyladenosine), I (inosine), Cm (2′-*O*-methylcytidine), f^5C (5-formylcytidine), $m^2{}_2G$ (*N2,N2*-dimethylguanosine), OHyW (hydroxywybutosine), τm^5U (5-taurinomethyluridine), mcm^5s^2U (5-methoxycarbonylmethyl-2-thiouridine), D (dihydrouridine), Ψ (pseudouridine), m^7G (7-methylguanosine), Q (queuosine), and X (various modifications).

contribute by increasing the binding energies of T54-m^1A58 and 13–22–m^7G46 interactions [22].

Regulation of tRNA local structure

In addition to the stabilization of the overall tRNA structure, tRNA modifications regulate the local tRNA structure in two main ways: (a) strengthening/weakening the rigidity of the RNA helical structure and (b) shaping the 'U-turn' anticodon loop structure.

Four types of tRNA modifications are known to affect RNA helix structural rigidity: (a) 2′-*O*-methylation (Nm, N = any bases), (b) 2-thiolation of xm^5s^2U derivatives [5-methoxycarbonylmethyl-2-thiouridine (mcm^5s^2U), or τm^5s^2U in humans], (c) pseudouridine, and (d) dihydrouridine. The ribose ring can form two conformations, namely the C2′-endo conformation and C3′-endo conformation (Fig. 1D) [23]. When the ribose is in the C2′-endo form, the base and the 2′-hydroxyl group are in proximity (Fig. 1D). In the presence of Nm or 2-thiolation of xm^5s^2U derivatives, the C3′-endo form is predominant due to the increased steric repulsion between the even enlarged 2′ hydroxyl moiety and the base [23,24].

Pseudouridine (Ψ) is a C–C glycosidic isomer of uridine (Fig. 1C). This isomerization exposes the *N1* hydrogen, which can bridge with the phosphate backbone via a water molecule. Pseudouridylation improves base stacking in a helical environment, favoring the ribose C3′-endo conformation [25,26]. These Ψ-mediated structural stabilizations are the likely cause of the tRNA thermal stabilization observed in the presence of Ψ27, Ψ39, or Ψ50 [27–29]. Together, Nm, the 2-thiolation of xm^5s^2U derivatives, and Ψ can stabilize the RNA helical structure in the tRNA arm or codon–anticodon minihelix. Many of these modifications are essential for health. For instance, mutations in the genes of the enzymes responsible for 2-thiolation of xm^5s^2U derivatives, Gm34, or Ψ39 result in microcephaly and/or intellectual disability and/or nephropathy [30–32].

In contrast to Nm, 2-thiolation, and Ψ that stabilize the RNA helical structure, dihydrouridine (D) destabilizes the helical structure. Indeed, D is observed only within loops, namely at D loop positions 16, 17, 20, 20a, and variable loop position 47 (Fig. 2). D is formed by the addition of two hydrogens to the C5=C6 bond (Fig. 1C), which break the planar structure of the uridine base, resulting in a predominance of the ribose C2′-endo conformation over the C3′-endo conformation [33].

Another important structural role of tRNA modifications is the shaping of a defined 7-nt anticodon loop structure, called the 'U-turn'. Different tRNAs have different anticodon loop sequences. However, every anticodon loop entering the ribosomal A-site must have a similar conformation to allow for efficient protein synthesis. This is accomplished by making the tRNA anticodon loops of all tRNAs adopt a highly similar U-turn structure with the help of tRNA modifications, especially at positions 34 and 37, which are heavily modified. Position 34 has complex modifications, such as mcm^5s^2U, 5-taurinomethyluridine (τm^5U), or Q, and position 37 also often possesses complex modifications, including i^6A, t^6A, 2-methylthio-*N6*-threonyl carbamoyladenosine (ms^2t^6A), and hydroxywybutosine (OHyW; Figs 1C–3). tRNA modifications at positions 34 and 37, such as mcm^5U, mcm^5s^2U, Q, *N6*-isopentenyladenosine (i^6A), t^6A, and m^1G, increase the stacking interactions of bases and restrict movement of the anticodon loop [34]. In addition, many modifications in the anticodon loop prevent unwanted intraloop base pairing that would disrupt the U-turn structure [35]. Another very important function of tRNA modification at positions 34 and 37 is to enable precise and efficient decoding, which will be discussed in a later section.

Inhibition of RNase-mediated degradation

In response to stress-inducing stimuli, human cytoplasmic tRNAs are frequently cleaved within the

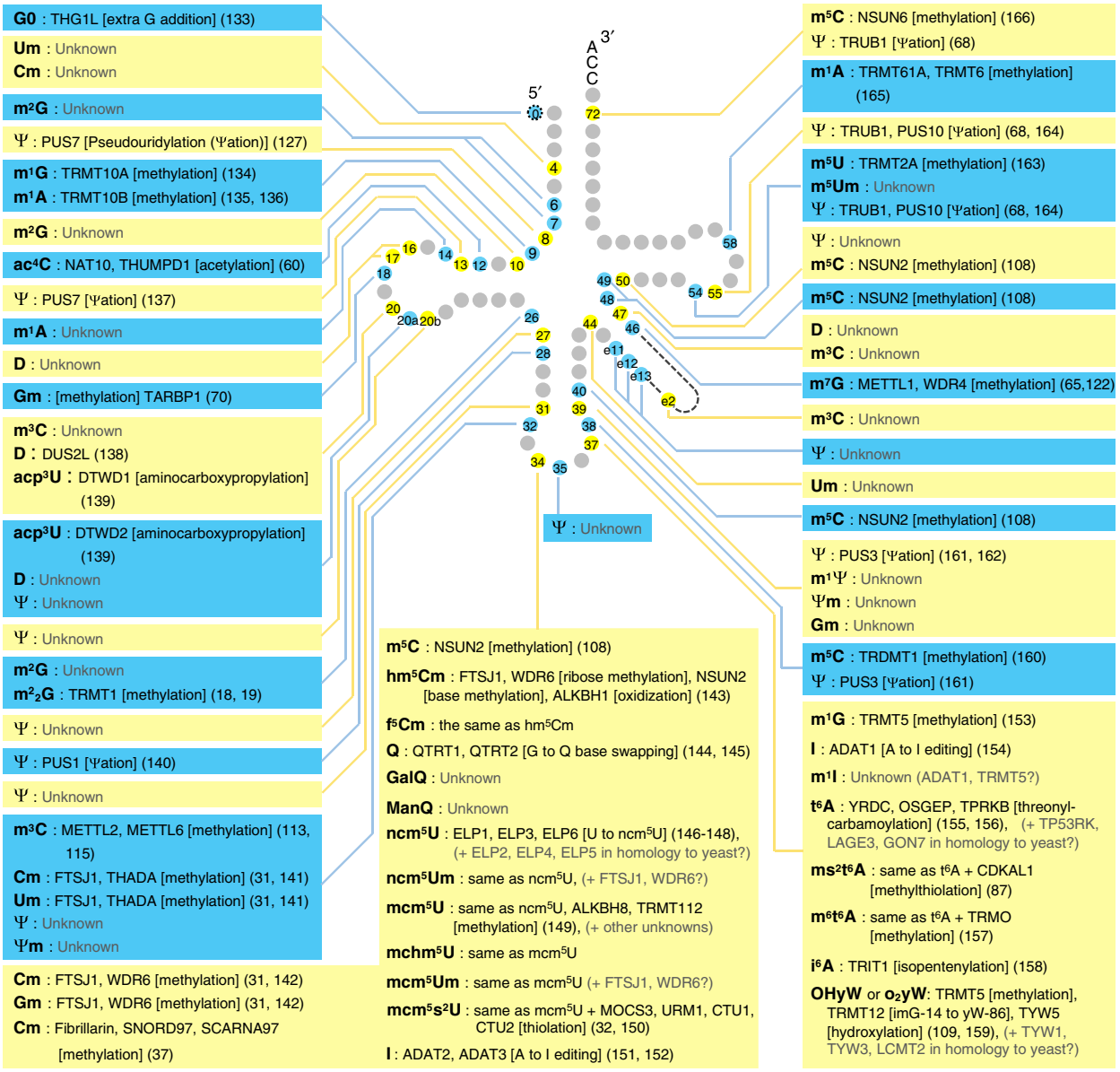

Fig. 2. Mammalian cytoplasmic tRNA modifications and modification enzymes. The name of the modification enzyme, the reaction the enzyme is responsible for (in brackets), and the reference (in parentheses) is written next to the species of tRNA modification. Insights are derived mostly from human studies and in part from other mammalian species studies. Note that the strength of evidence varies between different studies, ranging from checking only that the protein is necessary for modification to completely confirming that the protein is both necessary and sufficient for the modification. For the structures of modifications not depicted in Fig. 1, please refer to the RNA Modification Database (https://mods.rna.albany.edu). Abbreviations not described in Fig. 1: G0 (Guanosine added post-transcriptionally), Um (2′-*O*-methyluridine), m^2G (*N2*-methylguanosine), m^1G (1-methylguanosine), ac^4C (*N4*-acetylcytidine), Gm (2′-*O*-methylguanosine), m^3C (3-methylcytidine), acp^3U (3-(3-amino-3-carboxypropyl)uridine), Ψm (2′-*O*-methylpseudouridine), m^5C (5-methylcytidine), hm^5Cm (5-hydroxymethyl-2′-*O*-methylcytidine), f^5Cm (5-formyl-2′-*O*-methylcytidine), GalQ (galactosyl-queuosine), ManQ (mannosyl-queuosine), ncm^5U (5-carbamoylmethyluridine), mcm^5U (5-methoxycarbonylmethyluridine), $mchm^5U$ (5-(carboxyhydroxymethyl)uridine methyl ester), mcm^5Um (5-methoxycarbonylmethyl-2′-*O*-methyluridine), m^1I (1-methylinosine), m^6t^6A (*N6*-methyl-*N6*-threonylcarbamoyladenosine), o^2yW (peroxywybutosine), $m^1Ψ$ (1-methylpseudouridine), m^5U (5-methyluridine), and m^5Um (5,2′-*O*-dimethyluridine).

anticodon loop by angiogenin, an endoribonuclease belonging to the RNaseA family [36]. Several anticodon tRNA modifications, namely Cm34 and Q34, are known to prevent angiogenin-mediated cleavage [37,38]. Cm34 in $tRNA^{eMet}$ is incorporated by the Fibrillarin/snoRNA machinery and inhibits angiogenin-

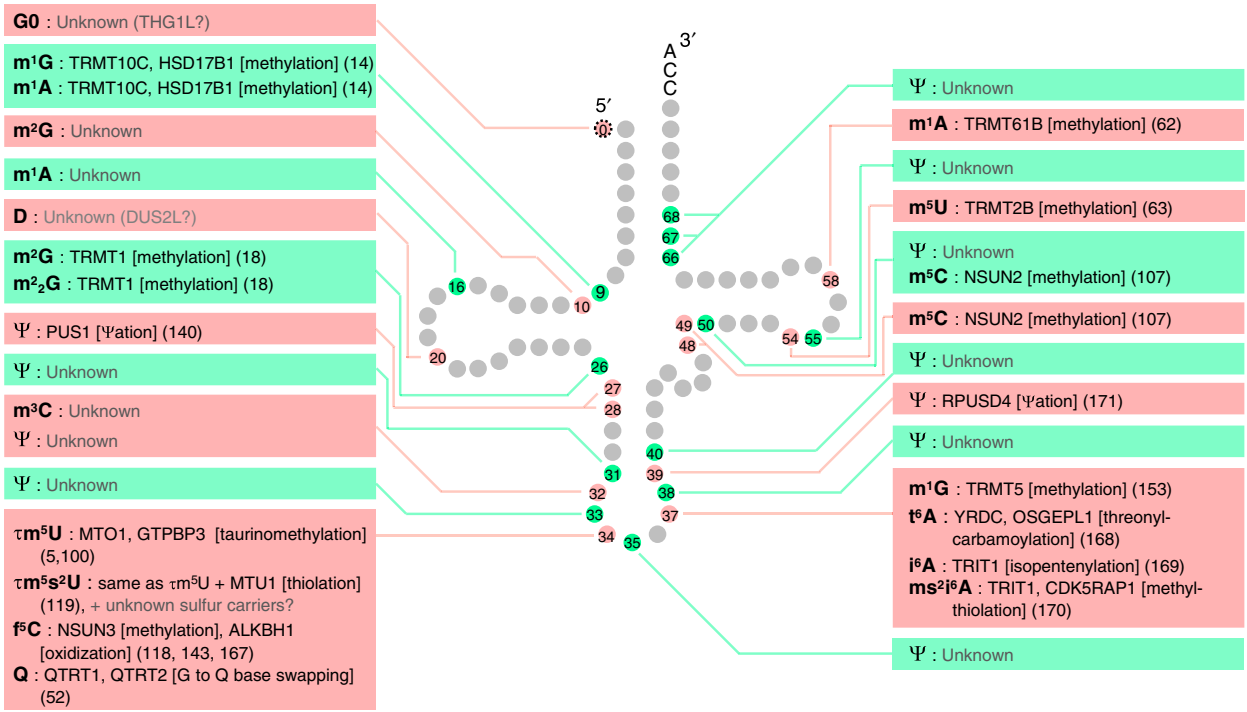

Fig. 3. Human mitochondrial (mt) tRNA modifications and modification enzymes. The name of the modification enzyme, the reaction the enzyme is responsible for (in brackets), and the reference (in parentheses) is written next to the species of tRNA modification [52]. Note that the strength of evidence varies between different studies, ranging from checking only that the protein is necessary for modification to fully confirming that the protein is both necessary and sufficient for the modification. For the structures of modifications not depicted in Fig. 1C, please refer to the RNA Modification Database (https://mods.rna.albany.edu). The secondary structures of many mt tRNAs are different from the canonical cloverleaf structure in three ways [13,46,224–227]: (a) mt $tRNA^{Ser(AGY)}$ lacks the entire D loop, (b) mt $tRNA^{Ser(UCN)}$ lacks U8 and has a small D loop, a small variable loop, and an extended anticodon stem, and (c) several mt tRNAs do not have canonical D loop/T loop interactions and instead have alternative interactions. Abbreviations not described in Figs. 1 and 2: τm^5s^2U (5-taurinomethyl-2-thiouridine) and ms^2i^6A (2-methylthio-*N6*-isopentenyladenosine).

mediated cleavage [37]. This protection is provided presumably because 2′-*O*-methylation precludes the deprotonation of the ribose 2′ OH, which is a process needed for RNases such as angiogenin to cut the phosphate backbone. The mechanism of how Q34 prevents angiogenin-mediated cleavage remains unelucidated. In addition, NOP1/NOP2/Sun domain family member 2 (NSUN2)-mediated m^5C formation inhibits angiogenin-mediated tRNA cleavage. This protection from angiogenin is likely due to reduced angiogenin-binding affinity in the presence of m^5C [39], although it is not yet clear which of the m^5C modifications at positions 34, 48, 49, and 50 inhibits angiogenin. Mutations in the *NSUN2* gene cause microcephaly, intellectual disability, and growth retardation [40,41]; m^5C-mediated tRNA protection from angiogenin is important for health, as cellular stresses in the brain caused by m^5C deficiency can be rescued by inhibiting angiogenin [39].

Compared to the endonucleolytic tRNA cleavage mechanism, the exonucleolytic tRNA decay mechanism is poorly characterized in humans. In yeast, when $tRNA^{iMet}$ lacks m^1A58, the tRNA is subjected to 3′–5′ decay by the TRAMP complex [42], and $tRNA^{Val}_{AAC}$ lacking both m^7G46 and m^5C (at positions 34, 40, 48, and/or 49) is subjected to 5′–3′ decay by the rapid tRNA degradation (RTD) pathway [43]. In humans, although such exonucleolytic pathways have not been formally characterized, the existence of similar pathways has been suggested [44]. The molecular characterization of human exonuclease-mediated decay pathways for hypomodified tRNA is awaited.

Function of tRNA modifications: decoding

The 20 universal amino acids are encoded by 61 codons ($4^3 = 64$ codons, minus three stop codons). Most of these codons are organized in codon family boxes, in which synonymous codons code for the same amino acid. In the decoding process, the codon triplet

(codon positions 1, 2, and 3) base pairs with the three anticodon bases of the tRNA in positions 36, 35, and 34, respectively (Fig. 1A). Codon positions 1 and 2 base pair with tRNA positions 36 and 35 in normal Watson-Crick base pairs (A–U, G–C). In contrast, the formation of a nonstandard base pair between the 3rd base of the codon and tRNA position 34 (the so-called wobble base pair) is permitted [45]. Consequently, one tRNA molecule can often decode several synonymous codons. For example, human mitochondrial tRNA with an unmodified U at position 34 (U34) decodes four synonymous codons in a four-codon box (e.g., one $tRNA^{Gly}$ decodes GGA, GGU, GGG, and GGC codons to incorporate glycine) [46].

The modification at tRNA position 34 ensures restricted or, sometimes, relaxed codon recognition by the tRNA anticodon [11,47]. An xm^5s^2U modification at position 34, such as human mcm^5s^2U (Fig. 1C) or τm^5s^2U, largely fixes its ribose in the C3′-endo form and leads to preferential base pairing with A- or G-ending codons and not to U- or C-ending codons [46,48,49]. Queuosine (Q, Fig. 1C) and its sugar-added derivatives (ManQ, GalQ) are present at position 34 of cytoplasmic $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$, and $tRNA^{Asp}$; these base pair with U- or C-ending codons and not A- or G-ending codons. Q prevents frameshifting and promotes efficient translation of these codons, although the precise mechanism is unknown [50,51]. Q34 is also present in mitochondrial tRNAs and likely promotes translation of tyrosine in mitochondria [52]. Inosine (I, Fig. 1C) is synthesized by the post-transcriptional deamination of adenosine (A), and I at position 34 (I34) expands tRNA decoding capacity. I34 facilitates tRNA base pairing not only with U-ending codons but also with C- and A-ending codons [45]. Similarly, 5-formylcytidine (f^5C, Fig. 1C) and 5-hydroxymethylcytidine (hm^5C) also expand tRNA decoding capacity. Although an unmodified C34 can only decode G-ending codons, f^5C or hm^5C can decode both A- and G-ending codons [53].

tRNA position 37, located at the 3′ side of the anticodon, often possesses a bulky modification, such as OHyW or ms^2t^6A (Figs 1–3). These position 37 modifications play a critical role in the stabilization of codon–anticodon pairing and maintain the reading frame by increasing base-stacking interactions and/or preventing unwanted base pairing within the anticodon loop [34,50,54–56]. Aberration of tRNA modifications in anticodon positions 34 and 37 induces various tRNA modopathies, including brain disorders, mitochondrial diseases, diabetes, and cancer (Table 1), which will each be discussed in detail in later sections.

Other functions of tRNA modifications and modifying enzymes

In addition to tRNA stabilization and decoding, some tRNA modifications and tRNA modifying enzymes perform additional functions. Such functions should not be disregarded, as a disease mutation in a tRNA modification enzyme gene may disrupt these additional functions and drive pathogenesis.

First, a tRNA modification can serve as the recognition determinant for another tRNA modification enzyme. For example, Cm32 modification of cytoplasmic $tRNA^{Phe}$ promotes the formation of OHyW [31]. In yeast, Cm32 and m^1G37 are required for Gm34 formation, and Gm34 is required for yW37 formation [57]. The same recognition mechanisms might also work in the human OHyW37 formation. In addition, the Q34 modification of cytoplasmic $tRNA^{Asp}$ promotes the efficient modification of m^5C38 [51].

Second, a tRNA modification enzyme can function not only as a tRNA modification enzyme but also as a modification enzyme for different RNA species. For example, NAT10, a cytoplasmic tRNA acetyltransferase, also acetylates 18S rRNA and various mRNAs [58–60]. Mitochondrial (mt) tRNA methylases TRMT2B and TRMT61B also methylate mt 12S rRNA and mt 16S rRNA, respectively [61–63]. In addition, methyltransferase-like 1 (METTL1), a cytoplasmic tRNA m^7G46 methylase, also methylates the precursor of let-7 microRNA [64,65].

Third, a tRNA modification enzyme can sometimes perform two functions, and one function can be completely different from tRNA modification. A prominent example is TRMT10C, a mt tRNA m^1A9/m^1G9 methylase that also functions as an essential component of mt RNaseP, an endoribonuclease complex that cleaves the 5′ end of a tRNA from the precursor RNA [14,66]. Additionally, TRUB1, a cytoplasmic tRNA pseudouridylase for Ψ54, Ψ55, and Ψ72, binds to the let-7 microRNA precursor but does not modify it. Instead, TRUB1 promotes cleavage of the let-7 microRNA precursor by enhancing the interaction between the let-7 microRNA precursor and an endoribonuclease complex [67,68]. In yeast, Trm2 functions not only as a tRNA m^5U54 methylase but also as a tRNA chaperone [69]. Whether the human homologs of Trm2, namely, TRMT2A and TRMT2B, have similar tRNA chaperone activity is still unknown.

Fourth, a tRNA modification can affect immune responses. Transfection of human total tRNA deficient in Gm18 induces innate immune responses by stimulating Toll-like receptors TLR7/8, whereas total tRNA of wild-type cells does not stimulate immune responses [70]. In

Table 1. tRNA modopathies. The tRNA modopathy genes are ordered by the nucleoside position of the tRNA modification that the gene product incorporates. Note that the strength of evidence varies between different studies, ranging from simply correlation studies to thorough investigation studies using both human patient cells and mouse disease models. The severity of disorders may vary between patients with mutations in the same gene. In the 'Modification' column, an 'm' is added to the position number of mitochondrial tRNA modifications. tRNA modifications for which modifying enzymes have not been formally investigated in humans but are commonly predicted by researchers in the field are indicated with a question mark. For cancers in the 'tRNA modopathy' column, a note is written in parenthesis to indicate the status of the modification enzyme in the cancer tissue. In the 'B', 'K', 'S', 'M', and 'C' columns, these letters indicate the occurrence of most frequently occurring tRNA modopathies or symptoms in the forms of brain-related disorders (B), kidney-related diseases (K), short stature (S), mitochondrial diseases (M), and cancer (C). Abbreviations: autism spectrum disorder (ASD), intellectual disability (ID), amyotrophic lateral sclerosis (ALS).

Gene	Modification (position)	Enzymatic activity	tRNA modopathy	B	K	S	M	C	Ref
THG1L	G (0)	Extra G addition	Microcephaly, cerebellar ataxia, ID, nephropathy, short stature	B	K	S			[172–174]
PUS7	Ψ (8, 13)	Pseudouridylation	Microcephaly, ID, ASD, aggressive behavior, short stature	B		S			[137,175,176]
TRMT10A	m^1G (9)	Methylation	Microcephaly, ID, diabetes, short stature	B		S			[134]
TRMT10C	m^1G, m^1A (9m)	Methylation	Lactic acidosis, hypotonia, polymicrogyria, deafness, early death	B			M		[15]
HSD17B10	m^1G, m^1A (9m)	Partner protein of TRMT10C	Neurodegeneration, cardiomyopathy, early death	B			M		[16]
NAT10	ac^4C (12)	Acetylation	Colon cancer (mislocalized), liver cancer (high expression)					C	[177,178]
THUMPD1	ac^4C (12)	Partner protein of NAT10	Breast cancer (mislocalized, high expression)					C	[179]
TARBP1	Gm (18)	Methylation	Liver/skin cancers (high expression)					C	[180,181]
DUS2L	D (20)	Hydrogen addition to U	Lung cancer (high expression)					C	[182]
TRMT1	$m^2{}_2G$ (26, 26m) m^2G (26?, 26m)	Methylation	Microcephaly, ID	B					[20]
PUS1	Ψ (27, 28, 30, 27m, 28m)	Pseudouridylation	Mitochondrial myopathy, sideroblastic anemia (MLASA)				M		[140,183]
METTL6	m^3C (32)	Methylation	Breast cancer (gene amplification)					C	[184]
THADA	Nm (32)	Partner protein of FTSJ1	Diabetes						[185]
FTSJ1	Nm (32, 34)	Methylation	ID	B					[31,186]
NSUN2	m^5C (34, 48, 49, 50, 48m, 49m, 50m)	Methylation	ID, Dubowitz-like syndrome, short stature, breast cancer (high expression)	B		S		C	[40,41,187]
ADAT3	I (34)	A to I editing	ID, strabismus	B					[188]
QTRT1	Q (34, 34m)	G to Q base swapping	Colon cancer (absence)					C	[78,80]
ELP1	ncm^5U (34)	U to ncm^5U (as a component of catalytic ELPS complex)	Familial dysautonomia, male infertility, skin cancer (high expression)					C	[77,148,189]
ELP2	ncm^5U? (34)	U to ncm^5U modification	ID, ASD	B					[190,191]
ELP3	ncm^5U (34)	U to ncm^5U modification	ALS, skin/breast cancers (high expression)					C	[77,192,193]
ELP4	ncm^5U? (34)	U to ncm^5U modification	ID, ASD	B					[194]
ELP5	ncm^5U? (34)	U to ncm^5U modification	Diabetes						[195]
ALKBH8	mcm^5U (34), $mchm^5U$ (34)	cm^5U to mcm^5U, then to $mchm^5U$ modification	ID, bladder cancer (high expression)	B				C	[196,197]
CTU1	mcm^5s^2U (34)	2-thiolation (with CTU2)	Skin/breast cancers (high expression)					C	[77,192]
CTU2	mcm^5s^2U (34)	2-thiolation (with CTU1)	Microcephaly, ID, nephropathy, ambiguous genitalia, short stature, skin/breast cancers (high expression), early death	B	K	S		C	[32,77,192]

Table 1. (Continued).

Gene	Modification (position)	Enzymatic activity	tRNA modopathy	B	K	S	M	C	Ref
MTO1	τm^{5}U (34m)	Taurinomethylation (with GTPBP3)	Hypertrophic cardiomyopathy, lactic acidosis, ID, short stature, early death	B		S	M		[98,198]
GTPBP3	τm^{5}U (34m)	Taurinomethylation	MELAS, ID, hearing loss, short stature, early death	B		S	M		[99]
MTU1	τm^{5}s^{2}U (34m)	Thiolation	Hepatopathy, lactic acidosis, Leigh syndrome, hearing loss, early death	B			M		[199–203]
NSUN3	f^{5}C (34m)	Methylation (followed by oxidization by ALKBH1)	Microcephaly, seizure, lactic acidosis, muscle weakness, short stature, 5-AZA-resistant leukemia (high expression)	B		S	M	C	[117,118,204]
ALKBH1	hm^{5}Cm (34), f^{5}Cm (34), f^{5}C (34m)	Oxidization (m^{5}C to f^{5}C, m^{5}Cm to hm^{5}Cm to f^{5}Cm)	Gastric cancer (low expression)					C	[205]
ADAT1	I (37)	A to I editing	Coronary artery disease						[206]
TRMT5	m^{1}G (37, 37m), OHyW? (37)	Methylation	Cardiomyopathy, lactic acidosis, demyelinating neuropathy, renal tubulopathy, cirrhosis, short stature	B	K	S	M		[207]
TRMT12	OHyW (37)	imG-14 to yW-86	Colon cancer (low expression)					C	[54]
TYW3	OHyW? (37)	yW-86 to yW-72?	ALS						[208]
LCMT2	OHyW? (37)	OHyW-72 to OHyW?	Colon cancer (frameshift)					C	[111]
YRDC	t^{6}A (37, 37m)	Threonylcarbamoylation of A	Microcephaly, nephropathy, short stature, liver cancer (high expression), early death	B	K	S		C	[155,209]
OSGEP	t^{6}A (37)	Threonylcarbamoylation of A	Microcephaly, nephropathy, short stature, early death	B	K	S			[156]
TP53RK	t^{6}A? (37)	Threonylcarbamoylation?	Microcephaly, nephropathy, short stature, early death	B	K	S			[156]
TPRKB	t^{6}A (37)	Threonylcarbamoylation of A	Microcephaly, nephropathy, short stature, early death	B	K	S			[156]
LAGE3	t^{6}A? (37)	Threonylcarbamoylation?	Microcephaly, nephropathy, short stature, early death	B	K	S			[156]
GON7	t^{6}A? (37)	Threonylcarbamoylation?	Microcephaly, nephropathy	B	K				[155]
CDKAL1	ms^{2}t^{6}A (37)	Methylthiolation of t^{6}A	Diabetes						[104]
TRIT1	i^{6}A (37, 37m)	Isopentenylation of A	Microcephaly, ID, cardiomyopathy, lung cancer (low expression), short stature	B		S	M	C	[81,210]
CDK5RAP1	ms^{2}i^{6}A (37m)	Methylthiolation of i^{6}A	Glioma (high ms^{2}i^{6}A)					C	[211]
TRDMT1	m^{5}C (38)	Methylation	Gastric cancer (SNP association)					C	[212]
PUS3	Ψ (38, 39)	Pseudouridylation	Microcephaly, ID, nephropathy, short stature	B	K	S			[30,162,213]
TRMT44	Um? (44?)	Methylation	Partial epilepsy with pericentral spikes	B					[214]
METTL1	m^{7}G (46)	Methylation	Multiple sclerosis						[215]
WDR4	m^{7}G (46)	Methylation	Microcephaly, ID, nephropathy, short stature	B	K	S			[216,217]
TRMT2A	m^{5}U (54)	Methylation	Breast cancer (high expression)					C	[218]
PUS10	Ψ (54, 55)	Pseudouridylation	Crohn's disease, celiac disease						[219]
TRMT6	m^{1}A (58)	Partner protein of TRMT61A	Colon cancer (frameshift), liver cancer (high expression)					C	[111,220]
TRMT61A	m^{1}A (58)	Methylation	Bladder cancer (high expression)					C	[221]
TRMT61B	m^{1}A (58m)	Methylation	Breast cancer (High expression)					C	[222]

addition, whereas bacterial tRNA usually induces interferon-α secretion from human peripheral blood mononuclear cells, bacterial $tRNA^{Tyr}$ is not immunostimulatory because bacterial $tRNA^{Tyr}$ has a Gm18 modification that functions as an antagonist of TLR7 [71,72].

Fifth, tRNA modifications affect precursor tRNA splicing in some eukaryotes. In several precursor tRNAs, an intron in the anticodon stem-loop is post-transcriptionally removed by tRNA splicing. In *Trypanosoma brucei*, unusual RNA modifications, namely G–A editing and A–U editing, within the intron of pre-$tRNA^{Tyr}_{GUA}$ promote intron removal from pre-$tRNA^{Tyr}_{GUA}$ [73]. Conversely, tRNA splicing can affect tRNA modification. The intron in human pre-$tRNA^{Leu}_{CAA}$ needs to be removed for NSUN2-mediated m^5C34 formation [74]. Thus, aberration of various tRNA-related pathways should not be overlooked as a potential cause of tRNA modopathies.

tRNA modification enzyme genes

To understand how tRNA modopathies are caused by aberrations in tRNA modification, identification of modification enzymes is essential, as tRNA modopathies are often caused by mutations in tRNA modification enzyme genes. To the best of our knowledge, 43 different types of tRNA modifications are incorporated into human tRNA molecules by at least 73 enzymes and their partner proteins. Mammalian cytoplasmic tRNA modifications and their modification enzymes are shown in Fig. 2, and human mitochondrial tRNA modifications and their responsible enzymes are shown in Fig. 3.

tRNA modopathies

Mutations or expression changes in 54 tRNA modification enzymes and their partner proteins are known as the direct, or strong, candidate causes of various tRNA modopathies. In addition to a previous study that compiled human tRNA modifications and modopathies [75], we added many of the latest insights and provided more detailed information to the list of tRNA modopathies (Table 1). This number represents 72% of the 75 modification proteins (73 confirmed proteins or strong candidates, plus two weak candidates) and demonstrates the biological importance of tRNA modifications. The organ that is most frequently affected by tRNA modification deficiencies is the brain. Of the 54 tRNA modopathy-associated proteins, dysfunction of 28 proteins can cause or are associated with brain disorders (Table 1). Relatively severe brain disorders, such as microcephaly, are usually associated with intellectual disability and often associated with kidney disorders and/or short stature (Table 1). Relatively moderate brain disorders, such as intellectual disability or autism spectrum disorder, often occur without other apparent symptoms.

Our compilation shows that aberrations of 24 tRNA modification enzymes cause or are associated with cancer (Table 1). Cancer is often associated with a high rate of tRNA modification or high expression of tRNA modification enzymes. For example, mcm^5s^2U34 is necessary for the efficient translation of the AAA, GAA, and CAA codons, and high mcm^5s^2U34 is required for melanoma cells to survive [76]. The hypoxia-inducible factor 1-alpha (HIF1α) protein, which is enriched with these codons, requires the mcm^5s^2U34 modification enzymes ELP3, cytoplasmic tRNA 2-thiolation proteins 1, and 2 (CTU1 and CTU2) to be efficiently translated and to exert HIF1α-dependent metabolic reprogramming in melanoma [77]. In contrast, in several cases, a lower modification rate, including modifications such as i^6A, OHyW, or Q (Table 1), is associated with or sometimes directly promotes cancer formation [54,78–81]. The mechanism by which OHyW hypomodification causes colon cancer is described in a later section [54]. Compared to other tRNA modopathies in which mutations are usually inherited from parent(s), cancer-causing aberrations usually occur after birth, making it more difficult to distinguish a cancer-causing aberration from a mere cancer-associated aberration. To formally show that the aberration of a tRNA modification enzyme gene (upregulation, downregulation, or mutation) causes cancer, it is necessary to show at least two things: (a) The aberration of the gene is associated with poor survival in cancer patients, and (b) the aberration of the gene in a cell line increases virulence (e.g., cell proliferation, metastasis, or drug resistance).

Mitochondrial aberrations cause dysfunction in high-energy demand organs such as the brain and heart, and these diseases are collectively called 'mitochondrial diseases'. Dysfunction of at least 9 mitochondrial tRNA modification enzymes causes mitochondrial diseases, comprising a major group of tRNA modopathies (Table 1) [82]. In addition to mutations in mitochondrial tRNA modification enzymes, numerous mt tRNA mutations result in mt tRNA modification deficiencies and mitochondrial diseases (MITOMAP, https://www.mitomap.org/MITOMAP).

Codon-specific translational aberration in tRNA modopathies

In contrast to the diseases caused by mutations in general translation factors such as eukaryotic initiation factor 2 subunit alpha (eIF2α) and eukaryotic

elongation factor 1 alpha-2, which decrease the overall translation rate, one feature of tRNA modopathies is their codon-specific pathogenic mechanisms. For example, the pathogenic mitochondrial (mt) DNA A3243G mutation (*mt tRNA$^{Leu(UUR)}$* mutation) specifically causes the hypomodification of the τm^{5}U34 modification of mt tRNA$^{Leu(UUR)}$, which specifically reduces the translation of the UUG codon, resulting in mitochondrial disease [83]. The lack of the mt tRNA-$^{Leu(UUR)}$ τm^{5}U34 modification, and not the tRNA mutation itself, is responsible for translational deficiency [84]. Of the 13 proteins translated in mitochondria, the translation of ND6 mRNA is specifically and markedly reduced in A3243G mutant cells [85], likely due to the enrichment of the UUG codon in ND6 mRNAs [84].

Anticodon modifications at positions 34 and 37 directly regulate decoding, and mutations in the modification enzymes affect translation in a codon-specific manner and cause various diseases, such as diabetes, neurodegenerative diseases, and mitochondrial diseases (Table 1). In yeast and nematodes, the loss of cytoplasmic tRNA mcm^{5}s^{2}U34 slowed translation specifically at the AAA, CAA, or GAA codons, inducing protein aggregation. The codon translation rates and protein homeostasis were restored in yeast by overexpressing mcm^{5}s^{2}U-less tRNA, showing that the optimal codon translation rate is critical for maintaining proteome integrity [86]. In mammals, as described in a previous section, melanoma cells require cytoplasmic tRNA mcm^{5}s^{2}U34 for the efficient translation of the AAA, GAA, and CAA codons to enable efficient translation of NAA codon-rich HIF1α and HIF1α-dependent metabolic reprogramming [77].

As another example of codon-specific translational aberration in tRNA modopathy, a loss of the tRNA$^{Lys}_{UUU}$-specific ms^{2}t^{6}A37 modification decreased the translation of lysine codons, causing unfolded-protein responses and inducing the onset of type 2 diabetes [87].

In yeast, several tRNA modifications are required for cell survival under stressed conditions [88]. For example, in response to H_2O_2 exposure, an increased m^{5}C34 level of tRNA$^{Leu}_{CAA}$, which decodes the UUG codon, is observed; among the 38 UUG-enriched mRNAs in yeast, the m^{5}C-dependent translation of ribosomal protein Rpl22a was especially required for the cells to survive under stress [89]. In another case, certain DNA damage response genes in yeast are enriched with codons that are decoded by tRNAs containing mcm^{5}U34 or mcm^{5}s^{2}U34; Trm9, an enzyme required for these modifications, is essential for yeast cells to survive through DNA damage [90]. If similar tRNA modification-dependent stress-response mechanisms are identified in mammals, it would expand our understanding of the role of tRNA modifications in health and disease.

Of the many tRNA modopathies, pathogenic mechanisms have been thoroughly elucidated in only a few. The next sections will describe the pathogenic mechanisms of four relatively well-understood tRNA modopathies.

Mitochondrial (mt) diseases caused by a deficiency in mt tRNA taurine modification at position 34

The first identified tRNA modopathies were mitochondrial (mt) diseases caused by deficiencies in taurine modifications. In healthy individuals, two taurine-containing modifications are present at position 34 in five mt tRNAs: τm^{5}U (Fig. 1C) in tRNA$^{Leu(UUR)}$ and tRNA^{Trp} and 5-taurinomethyl-2-thiouridine (τm^{5}s^{2}U) in tRNA^{Glu}, tRNA^{Lys}, and tRNA^{Gln} [91]. These taurine modifications promote accurate mitochondrial translation of A- and G-ending codons and prevent misreading of C- or U-ending codons [46].

Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) and myoclonus epilepsy associated with ragged red fibers (MERRF) are severe mitochondrial diseases with various symptoms, including muscle weakness and epilepsy [92,93]. A majority of MELAS patients carry an A3243G mutation in the mt *tRNA$^{Leu(UUR)}$* gene on mtDNA [83], and many MERRF patients carry an A8344G mutation in the mt *tRNA^{Lys}* gene on mtDNA [94]. mt tRNA$^{Leu(UUR)}$ with that MELAS mutation and mt tRNA^{Lys} with that MERRF mutation lack taurine modifications and show deficiencies in recognizing their cognate codons [95–97]. Moreover, the lack of a τm^{5}U modification, and not the tRNA mutation itself, is responsible for disruption of translation [84]. MELAS- or MERRF-associated pathogenic tRNA mutations are presumed to prevent tRNA recognition by taurine modification enzymes, but formal studies have not been conducted.

In addition to mutations in mt tRNA genes, various mutations in taurine modification enzyme genes, namely the *MTO1* and *GTPBP3* genes, are observed in mitochondrial disease patients [98,99]. The mitochondrial translation optimization protein 1 homolog (MTO1)-GTP-binding protein 3 (GTPBP3) complex uses 5,10-methylenetetrahydrofolate and taurine as metabolic substrates for τm^{5}U formation [5]. Patients with *MTO1* or *GTPBP3* mutations show diverse symptoms starting in infancy or early childhood,

including optic neuropathy and cognitive disability, with cardiomyopathy being the most frequent symptom. To understand the role of the taurine modification *in vivo* and to recapitulate the pathogenesis of mitochondrial disease, animal models have been generated [100] (Fig. 4A).

Whole-body *Mto1* knockout in mice was embryonic lethal at an early developmental stage (approximately E9.0). *Mto1* KO embryonic stem cells showed a > 80% reduction in mitochondrial protein synthesis, poor assembly and activity of mitochondrial respiratory complexes, increased lactate levels, and increased NADH/NAD^+ ratios. Interestingly, the total ATP level in KO cells was only slightly decreased compared with that in WT cells due to increased cytoplasmic ATP production by glycolysis, as evidenced by increased lactate levels and NADH/NAD^+ ratios. Heart-specific *Mto1* knockout mice developed normally during the embryonic stage but could not survive more than 24 h after birth and showed elevated expression of the heart failure marker genes *Anp* and *Bnp*.

Mto1 knockout cells underwent not only metabolic changes but also protein homeostasis changes [100] (Fig. 4A). In healthy cells, more than 1000 nucleus-encoded proteins are translated in the cytoplasm by cytoplasmic ribosomes and then efficiently transported into mitochondria from the cytoplasm [101]. In *Mto1* knockout cells, the transport of mitochondria-targeted proteins across the inner membrane was defective. The defective mitochondrial transport of proteins from the cytoplasm may be due to decreased mitochondrial inner membrane integrity and/or decreased mitochondrial ATP generation that may decrease ATP-driven transmembrane protein transport by mitochondrial Hsp70 proteins; these possibilities, however, need to be investigated further. As a consequence of decreased mitochondrial transport from the cytoplasm, mitochondria-targeted proteins formed cytoplasmic protein aggregates and induced a cytotoxic unfolded-protein response.

Intriguingly, tauroursodeoxycholic acid (TUDCA), a chemical chaperone that improves protein folding and prevents protein aggregation, suppresses protein aggregation and moderately improves respiratory activity in both cell cultures and tissue-specific *Mto1* knockout mice [100]. The safety of TUDCA has been proven in humans, and the effect of TUDCA has been tested in clinical trials for diseases such as diabetes and amyloidosis [102,103]. As a symptomatic therapy, future clinical assessments need to be performed in mitochondrial disease patients to investigate whether TUDCA can mitigate the symptoms of mitochondrial diseases.

Type 2 diabetes caused by a deficiency in CDKAL1-mediated thiomethylation of cytoplasmic $tRNA^{Lys}_{UUU}$ at position 37

Cdkal1 is a mammalian methylthiotransferase that synthesizes 2-methylthio-*N6*-threonylacarbamoyladenosine (ms^2t^6A, Fig. 1C) at position 37 of cytoplasmic $tRNA^{Lys}_{UUU}$ [87]. The ms^2 modification of t^6A37 stabilizes the interaction between $tRNA^{Lys}_{UUU}$ and its cognate codon AAG as well as AAA and increases the translation rate of these codons (Fig. 4B) [87]. Whole-genome association studies identified a number of genes associated with type 2 diabetes (T2D). Among these risk genes, *CDKAL1* is one of the most common genes across different ethnicities [104]. Among the various tRNA modifications, T2D caused by *CDKAL1* single nucleotide polymorphisms (SNPs) may affect the largest human population, as suggested by genome-wide association studies. Various SNPs in the *CDKAL1* gene influence the risk of T2D, and *CDKAL1* SNPs are associated with decreased insulin secretion but not peripheral insulin sensitivity [105].

To understand the pathophysiology and pathogenesis of T2D, pancreatic β-cell-specific *Cdkal1* KO mice were studied [87] (Fig. 4B). In β-cell-specific *Cdkal1* KO mice, a deficiency in ms^2t^6A caused the mistranslation of proinsulin Lys codons, one of which is present at the proinsulin processing site, resulting in improper proinsulin processing. The mice showed pancreatic islet hypertrophy, decreased insulin secretion, and impaired blood glucose control. Mistranslation was associated with the endoplasmic reticulum (ER) stress response, and the mice were hypersensitive to high-fat diet-induced ER stress. Consistent with this model, human proinsulin conversion was decreased in homozygous carriers of *CDKAL1* risk SNPs [106].

Neurodevelopmental disorder caused by a deficiency in Nsun2-mediated m^5C modification

Autosomal recessive mutations in the human *NSUN2* gene were found to cause intellectual disability, microcephaly, behavioral deficits, speech delay, unusual facies, and growth retardation [40,41]. NSUN2 is a 5-methylcytidine (m^5C) modification enzyme for m^5C at cytoplasmic tRNA positions 34, 48, 49, 50, and at mitochondrial tRNA positions 48, 49, and 50 [107,108]. *Nsun2* knockout mouse models and human cells obtained from Dubowitz-like syndrome individuals were studied as disease models in order to understand the pathophysiology and pathogenesis [39] (Fig. 4C). In human cells and mouse tissues without

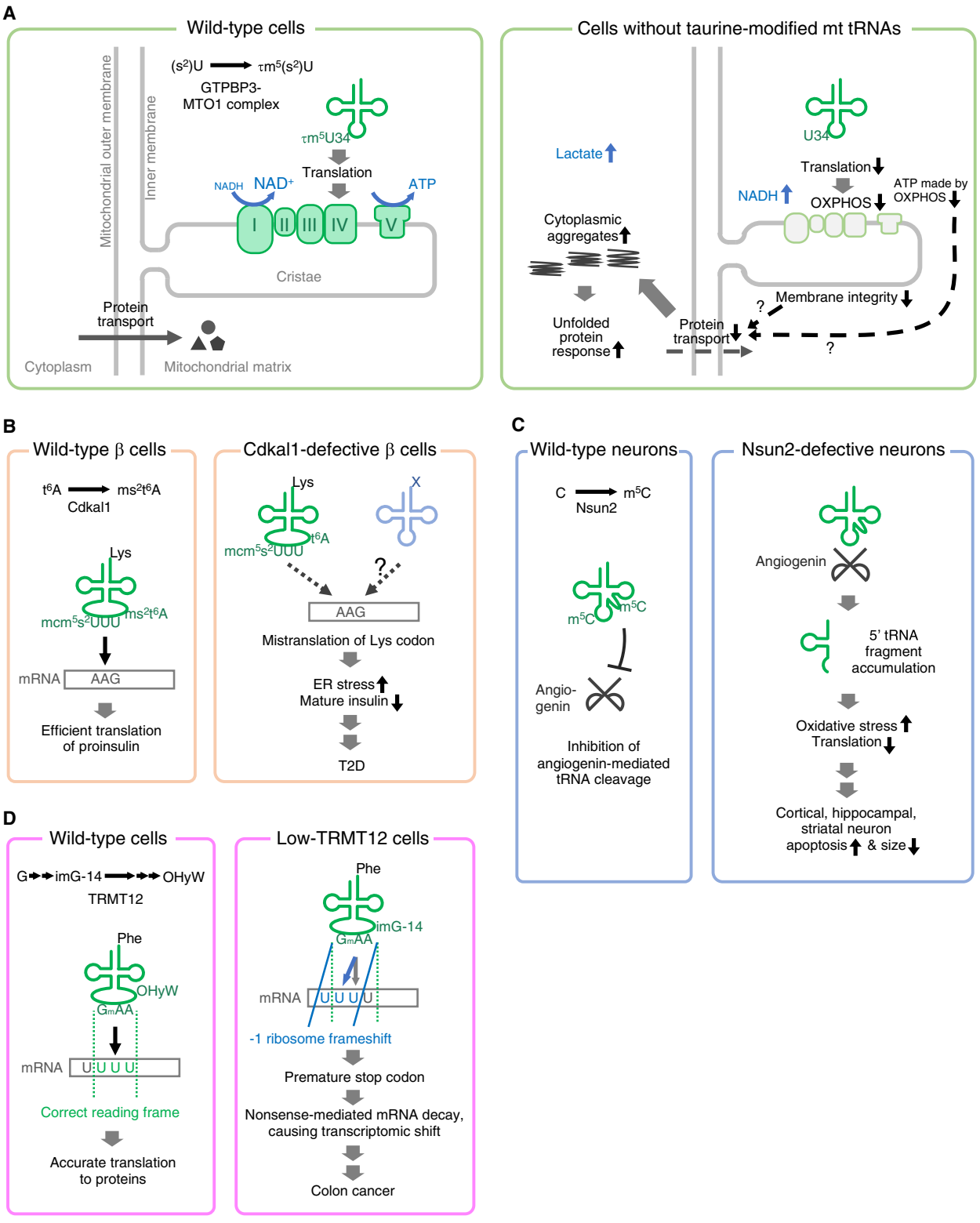

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Fig. 4. Pathogenic molecular mechanisms of tRNA modopathies. (A) Mitochondrial (mt) diseases caused by deficiencies of mt tRNA taurine modifications at position 34. The GTPBP3–MTO1 complex incorporates τm^5U34 modification into five mt tRNAs. Without the taurine modification, the translation rate of OXPHOS complex proteins declines, causing a metabolic shift as well as a proteostasis shift, especially affecting energy-demanding organs such as the brain and muscle. (B) Type 2 diabetes caused by a deficiency of CDKAL1-mediated thiomethylation of cytoplasmic $tRNA^{Lys}_{UUU}$ at position 37. Cdkal1 incorporates the ms^2 modification to t^6A37 of $tRNA^{Lys}_{UUU}$ and promotes translation of lysine from the AAA and AAG codons. Cdkal1 is especially important in pancreatic β cells, in which lysine-containing proinsulin is rapidly and massively translated upon glucose stimulus. (C) Neurodevelopmental disorder caused by a deficiency of NSUN2-mediated m^5C modifications. NSUN2 incorporates m^5C into several sites within tRNAs and inhibits angiogenin-mediated tRNA cleavage. NSUN2 deficiency induces the accumulation of 5′ tRFs, which evokes reduced translation rates and activated stress responses and is the cause of brain disorders, including microcephaly and intellectual disability. (D) Colon cancer caused by epigenetic loss of TRMT12-mediated OHyW modification of $tRNA^{Phe}$ at position 37. Epigenetic silencing of TYW2 is a cause of colon cancer via the loss of the OHyW37 modification, inducing a −1 ribosome frameshift to downregulate various mRNAs, conferring enhanced migration properties and epithelial-to-mesenchymal features to the cells.

functional NSUN2, an accumulation of 5′ tRNA fragments (tRFs) was observed. In the *Nsun2* KO mice, angiogenin-mediated tRNA cleavage resulted in 5′ tRF accumulation. The accumulation of 5′ tRFs reduced protein synthesis rates and activated stress responses and was accompanied by increased apoptosis of cortical, hippocampal, and striatal neurons. Importantly, the increased sensitivity of Nsun2-deficient brains to oxidative stress could be rescued by inhibiting angiogenin. To the best of our knowledge, this is the first case of detailed (although not complete) elucidation of the molecular pathogenesis of a brain tRNA modopathy. Further studies are needed to understand why the phenotypes of a whole-body *Nsun2* KO manifest mainly in the brain and not in other tissues.

Colon cancer caused by epigenetic loss of TRMT12 (TYW2)-mediated OHyW modification of $tRNA^{Phe}$ at position 37

Human $tRNA^{Phe}$ contains a $tRNA^{Phe}$-specific, bulky tRNA modification at position 37, called OHyW (Fig. 1C), or an oxidized derivative, peroxywybutosine (o_2yW). Similar to the well-characterized yeast wybutosine synthesis pathway, human wybutosine derivatives are presumed to be synthesized by six enzymes, namely TRMT5, TYW1, TRMT12 (TYW2), TYW3, LCMT2 (TYW4), and TYW5 [109,110]. A comprehensive analysis of the Cancer Genome Atlas revealed that the *TRMT12* promoter CpG island was methylated in many primary colorectal carcinoma cases, and *TRMT12* epigenetic inactivation was correlated with poor overall survival in patients with early-stage colorectal cancer [54]. In human cell lines, the *TRMT12* knockout induced the hypomodification of OHyW and increased −1 ribosome frameshifts at certain Phe codons. Those ribosome frameshifts created premature termination codons, resulting in transcript degradation via nonsense-mediated mRNA decay (Fig. 4D). Increased nonsense-mediated mRNA decay caused imbalances in the transcriptome, including in the mRNA levels of cell mobility-related genes, conferring migration properties and epithelial-to-mesenchymal features to TRMT12-deficient cells [54]. Interestingly, a frameshift mutation in the *LCMT2* (*TYW4*) gene, which encodes another enzyme presumed to be required to synthesize OHyW, is also found in colon cancers [111]. Thus, loss of OHyW derivatives might generally be involved in the formation of a subset of colon cancers.

Important questions to be addressed in order to understand the molecular pathogenesis of tRNA modopathies

Other than the four tRNA modopathies described above, the pathogenic mechanisms of most tRNA modopathies are poorly understood, especially in diseases caused by aberrant cytoplasmic tRNA modifications. In the next sections, we will raise and discuss four questions and problems that need to be addressed to elucidate the pathogenic mechanisms of various tRNA modopathies.

Mapping all human tRNA modifications

Due to accumulating knowledge regarding tRNA modification enzymes, we know which specific tRNA modification enzyme modifies specific positions of representative tRNA species. However, we do not have a complete understanding of which tRNA species are modified by each enzyme because we do not have a complete map of all the tRNA modifications of all tRNAs. Without knowing all the tRNA species that each enzyme modifies, it is difficult to understand the consequences of a specific modification enzyme dysfunction. A milestone study in this field is the complete identification of all mitochondrial tRNA modifications

in all 22 human mitochondrial tRNAs [52]. This work serves as an important foundation for understanding the molecular pathogenesis of mitochondrial tRNA modopathies. In contrast, the tRNA modifications of hundreds of cytoplasmic tRNA species transcribed in the nucleus are yet to be completely identified. Although the identification of all the modifications of all human cytoplasmic tRNA species requires tremendous work, such insight would greatly contribute to our understanding of the molecular pathogenesis of cytoplasmic tRNA modopathies.

Elucidation of the unidentified tRNA modification enzymes

Approximately 40 tRNA modification sites are modified by unknown enzymes (Figs 2 and 3). A fraction of these tRNA modifications are associated with strong candidate modification enzymes. This is because many of these modifications are located in the same positions as in yeast tRNAs, and the corresponding yeast tRNA modification enzymes have already been identified. In comparison with those in yeast, many additional tRNA modification enzymes exist in humans, many of which are likely generated by gene duplications from yeast homologs. Diversified human enzymes usually target different cytoplasmic tRNA species or different cellular compartments. For example, whereas only a single m^3C32 methylase, Trm140, exists in *Saccharomyces cerevisiae*, *Saccharomyces pombe* has two homologs, and humans have three homologs, namely METTL2, METTL6, and METTL8 [112,113]. The three human homologs are functionally differentiated. METTL2 synthesizes m^3C32 of cytoplasmic $tRNA^{Thr}$ and $tRNA^{Arg}$ [114]. METTL6 synthesizes m^3C32 of cytoplasmic $tRNA^{Ser}$ [115]. METTL8 localizes in mitochondria [116] and awaits investigation of whether it is responsible for m^3C32 of mitochondrial $tRNA^{Thr}$ and $tRNA^{Ser}$ [52]. m^3C32 is not present in $tRNA^{Arg}$ of *S. cerevisiae* but is present in human $tRNA^{Arg}$. Gene duplication and divergence expanded the substrate tRNA species. Even if a human tRNA modification is not conserved from yeast, it is still important to identify the responsible enzyme. Indeed, mt tRNA modifications such as τm^5U34 and f^5C34 are not present in yeast, but mutations in the responsible human enzymes cause severe mitochondrial diseases [98,99,117,118]. The cytoplasmic tRNA ms^2t^6A37 is also not present in yeast, but the dysfunction of the responsible human enzyme causes type 2 diabetes and affects a large human population [87,104].

tRNA modifications, such as GalQ34, ManQ34, and Nm39, are not associated with clear candidate enzymes. In addition, which pseudouridylases are responsible for Ψ at various positions remains unidentified.

Even for the tRNA modifications that are mediated by identified enzymes, it is possible that these enzymes may need additional partner proteins or upstream proteins to function. For instance, although the 2-thiolation of mitochondrial τm^5s^2U34 is catalyzed by MTU1 [119], how sulfur is carried to MTU1 is unknown. Analogous to the *S. cerevisiae* or *Escherichia coli* 2-thiolation pathways [120,121], it is likely that specific mitochondrial proteins relay sulfur from cysteine desulfurase to MTU1. Elps complex proteins (ELP1–6) and ALKBH8 are essential for forming cytoplasmic tRNA mcm^5U34 modification, but additional enzyme (s) are expected to form an intermediate cm^5U and remain unidentified [75].

Identifying how each tRNA modification affects mRNA translation and other steps of gene expression

To understand how a tRNA modopathy is caused, it is necessary to understand how translation is affected by the loss of tRNA modification. Although specific enzymes can modify various tRNAs, tRNA modifications often critically affect the translation of only a fraction of modified tRNAs. If tRNA modification deficiency affects anticodon:codon interactions or critically affects tRNA in other ways, tRNA modification deficiency would decrease the ribosome transition rate at the corresponding codon. Thus, techniques such as ribosome profiling would be useful for elucidating how the translation of a specific codon is affected following the loss of a tRNA modification enzyme. For instance, ribosome profiling revealed the codons at which ribosomes slow down upon loss of Wdr4, a protein required for m^7G46 modification [122]. Ribosome profiling can also identify which tRNAs are less frequently bound by ribosomes following the loss of specific species of tRNA modifications [123].

When the loss of a tRNA modification enzyme results in the decreased translation of an mRNA codon, one possibility is that the corresponding tRNA may degrade more easily without the modification. To investigate the effect of a tRNA modification on the tRNA steady-state level in an unbiased manner, quantification of the tRNA transcriptome is useful. As tRNAs are difficult to reverse-transcribe due to the presence of base pair-inhibitory modifications such as m^1A58, m^1G37, m^3C32, $m^2{}_2G26$, and m^1G9, it is helpful to use techniques such as demethylation via AlkB demethylase [124].

Recently, tRFs have become recognized to affect various steps of gene expression [125]. The generation of tRFs is affected by tRNA modifications such as Ψ, m^1G, Q, and m^5C [38,39,126,127]. Thus, we should not forget the possibility that some tRNA modopathies might be caused not only by dysfunctional tRNA but also by increased tRFs.

What causes the tissue specificity of tRNA modopathies?

Aberrations in various tRNA modification enzymes affect our body, often in tissue-specific or tissue-preferential ways (Table 1). The effects of mutations in important mitochondrial tRNA modification enzymes appear mostly in high-energy demand organs such as the heart and brain. This makes sense given that mitochondrial tRNA modification contributes to the translation of mitochondrial respiratory complex proteins used for ATP production.

Mutations in cytoplasmic tRNA modification enzymes most frequently affect the brain (Table 1); the mutations that affect brain development also often affect kidney development and overall body development (Table 1). We currently do not know why the brain is the most strongly affected organ. One clue may be that mutations in various other general translation regulatory proteins such as eIF2α cause neurological diseases, such as microcephaly, while having little effect to other tissues [128,129], and this is a highly similar phenotype seen in many tRNA modopathies. Perhaps neurons are extremely sensitive to relatively small changes in translational competency because these polarized cells require rapid and local protein synthesis for synaptic plasticity [128,129]. Neurons have long axons, and protein synthesis occurs not only in the cell body but also near synapses, which can be located at the end of long axons far from the cell body [130]. tRNA stability and translational efficiency may be especially important for translating proteins at such synaptic terminals, where tRNAs and ribosomes may not be transported from the cell body in abundance. Such possibilities merit investigation in order to understand the brain-biased phenotypes of various tRNA modopathies.

Although mutations in many cytoplasmic tRNA modification enzymes affect the brain, there are many exceptions. For example, *CDKAL1* SNPs are mainly correlated with type 2 diabetes, and CDK5 regulatory subunit-associated protein 1-like 1 (CDKAL1) dysfunction is not known to affect the brain, except for the role it plays in hormone biosynthesis in pituitary adenomas [131]. Why CDKAL1 mainly affects insulin biogenesis in pancreatic β-cells and not in other tissues is not fully understood. Dysfunction of β-cells in the context of CDKAL1 dysfunction, however, may at least in part be attributed to the heavy demand for the translation of proinsulin in β-cells. In a bacterial lysine translation reporter model, upon knockout of the bacterial CDKAL1-homolog, an increased translation rate led to decreased lysine translation fidelity [87]. As the translation of proinsulin comprises nearly 50% of total protein production upon glucose stimulation, and lysine is located at an important site within the proinsulin protein, it may be logical that β-cells are more affected by CDKAL1 deficiency than other tissues.

To understand the tissue specificity of tRNA modopathies, a global intertissue comparison of protein synthesis and tRNA status is essential. Some tissues may have a higher demand for the translation of specific mRNA codons, and some tissues may have a limited supply of tRNAs that translate those amino acids. The absence of a tRNA modification may greatly alter tRNA stability in different tissues, due to, for example, different expression levels of angiogenin (which can cleave the hypomodified tRNA anticodon loop) and its inhibitor RNH1 [39,132]. Therefore, to understand the tissue specificity of tRNA modifications, it would be useful to generate animal disease models and perform intertissue comparisons of the transcriptome (via RNA-seq) as well as protein synthesis (e.g., via ribosome profiling) between wild-type and disease model animals.

Concluding remarks

In recent decades, the identities and functions of many human tRNA modifications and the enzymes that cause these modifications have been elucidated. Moreover, tRNA modopathies resulting from aberrations in more than 50 tRNA modification enzyme genes have been discovered. Presently, the molecular pathogenesis of most tRNA modopathies remains unelucidated. In the next decade, the identification of all the tRNA modifications and modifying enzymes, as well as the intertissue comparison of protein synthesis in animal models, would elucidate these pathogenic mechanisms and provide evidence to support the development of treatments for these diseases.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

TC and KT wrote the review.

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